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14. ABSTRACT A mouse model of prostate cancer i.e. male C57/BL6 mice hemizygous for TRAMP and hetero or homozygous for tocopherol(TOC) transport protein (TTP) knockout was produced. C57/bl6 mice carrying the SV40 T antigen driven by the rat probasin promoter construct (TRAMP+) were bred with TTP knockout C57/bl6 mice, offspring with the desired sex and genotypes were raised on normal mouse chow. Animals were killed at multiple ages and GI tract, prostate pathology and plasma TOC contents were assessed. The results showed no difference in tumor development between the different TTP genotypes and their associated altered plasma levels of aT and gT. These strongly suggested that a reduction of plasma (and presumptively tissue, though unmeasured in this study) tocopherol achieved by manipulating the levels of TTP does not accelerate the course of prostate tumor formation. This raises questions regarding the possible effectiveness of tocopherol supplementation as treatment strategy for prostate cancer with possible next steps using direct supplemental feeding studies of both different tocopherols as well as these tocopherols' metabolites in a TRAMP model to assess their roles in the development and progression of TRAMP induced prostate tumors.					
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## **Introduction**

Prostate cancer is the most common tumor in U.S. men with nearly 30,000 deaths and more than 215,000 new cases expected. The growth and development of prostate cancer is initially androgen-dependent, and androgen ablation therapies have been the most common treatment for metastatic prostate cancer since the early 1940's [1, 2]. Whereas prostate cancer patients treated with androgen ablation therapy often go into remission, progression to androgen-independence commonly occurs within a few years [3]. Despite extensive efforts, clinical trials of both experimental and approved chemotherapeutic agents, the efficacy of chemotherapy has been seen as needing improvement [4]. Indeed, chemotherapeutic treatment of prostate cancer has an objective response rate of less than 10% and no demonstrated survival benefit in advanced disease [5]. Therefore, given the immense public health implications, identifying further effective chemopreventive and/or chemotherapeutic strategies against this disease would be extremely useful.

One such potential chemopreventative is the class of compounds collectively known as tocopherols which have attracted a great deal of recent clinical interest due to their widely recognized antioxidant properties [6]. The rationale for targeting tocopherol to prevent prostate cancer is that a link exists between inflammation, oxidation and cancer wherein inflammation imposes an oxidative stress [7] and there is increasing evidence that this plays an important role in prostate cancer development and/or progression [8, 9]. Finally evidence comes from the clinical studies which have shown that supplementation with micronutrient antioxidants such as  $\alpha$ -tocopherol ( $\alpha$ T) and Se decreases (albeit to a modest degree) the incidence of prostate cancer in humans [10] and reduced prostate cancer incidence by 63% compared to subjects given placebo, respectively [11]. The linkage of prostate cancer to the antioxidant  $\alpha$ T intake has also been documented by Heinonen and coworkers and by Chan and coworkers [12, 13].

However the presumption that increasing solely  $\alpha$ T intake, the treatment chosen for the SELECT human clinical trial, appears to conflict several recent epidemiological studies that have raised questions as to the exact identity of tocopherols responsible for the prostate cancer risk reductions noted [10, 14]. These epidemiological studies have reported that risk stratification by increasing  $\alpha$ T alone shows no prostate cancer risk reduction. Rather there appears a more complicated relationship wherein increased  $\alpha$ T levels are related to decreased prostate cancer risk but only in the context of "sufficient" levels of  $\gamma$ T. This then raises concerns as to the use of  $\alpha$ T only supplement [15]. A central element of this concern is it has been reported that increased  $\alpha$ T intake reduces  $\gamma$ T [16, 17] although interestingly the converse does not appear to be true, i.e. increased  $\gamma$ T intake does not drive down  $\alpha$ T, see for example [18]. Thus the potential for "perverse" effects as  $\gamma$ T may decline upon supplementation with only  $\alpha$ T and this may then actually increase prostate cancer risk.

## **Body**

The investigation undertaken was designed to further define tocopherol molecules/ molecular targets/mechanisms that mediate the effect of tocopherols on prostate cancer. To do so, we crossbreed mice bearing the TRAMP construct with a tocopherol transport protein (TTP) knockout transgenic mouse.

TRAMP mouse has become a popular preclinical model for studying chemoprevention of PCa as it exhibits similarities with human prostate cancer, including epithelial origin, progression from the PIN stage to adenocarcinoma, and metastasis by a transgene that is hormonally regulated by androgens. The prostate-specific expression of the SV40 large T/t antigen (Tag) leads to spontaneous evolution of intraepithelial hyperplasia and dysplasia, high grade PIN, and well-differentiated PCa by 10–12 weeks of age. increasingly well characterized model of human prostate cancer, the TRAMP mouse [19]. By using this animal model, we will facilitate the extrapolation of the data obtained to human prostate cancer. However the time course appears dependent on the genetics of the mice as the development of PC in C57/bl6 x C57/bl6 is significantly slower than that found in the more typically reported C57/bl6 x FVB crosses. However, these early prostatic lesions display a high degree of histological resemblance to human PIN and PCa and TRAMP mice have been used to demonstrate antitumorigenic effects of multiple compounds and treatments including green tea polyphenols [20], and dietary restriction [21, 22].

## **KEY RESEARCH ACCOMPLISHMENTS**

- Production of male TTP<sup>-/-</sup> TRAMP + mouse C57/bl6 x C57/bl6 model for prostate cancer.
- Long term observation of chow fed hetero (TTP<sup>+/-</sup>) and homozygote TTP<sup>-/-</sup> TRAMP + male mice
- Assessment of tumor development and plasma tocopherol (both  $\alpha$  and  $\gamma$ ) levels in relation to age of mouse.

## **REPORTABLE OUTCOMES**

### **Breeding**

Over the course of the project, we crossed TTPKO C57/bl6 males with TTPKO heterozygote females carrying TRAMP<sup>+</sup>, using this approach we obtained 255 births. Of those, 53% were male mice. Of the male mice, 48% were TRAMP<sup>+</sup>. Of the TRAMP<sup>+</sup> males, 60% were TTP heterozygotes while 40% were TTP homozygotes. This 60/40 split between TTP heterozygotes and homozygotes occurred in both males and females irrespective of TRAMP status. There were no statistical differences in the distribution of the ages amongst the homozygote and heterozygote TTP mice ( $p > 0.78$ )

### **Plasma tocopherol levels**

We analyzed the plasma obtained from both the heterozygote and homozygote TTP KO/Tramp males. The results are presented in Table 1 and are similar to those reported earlier by our laboratory [23, 24] and indicate that the plasma levels of  $\alpha$ T decline by 10X when TTP is completely removed and  $\gamma$ T declined as well going below the detection limit of the HPLC based assay in the case of the TTP<sup>-/-</sup> TRAMP<sup>+</sup> animals.

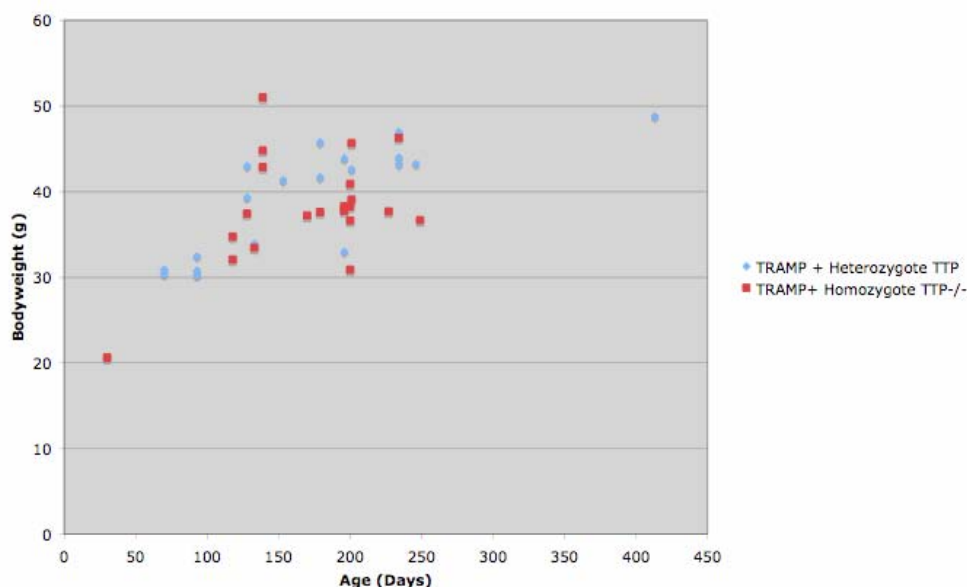
Table 1.

	$\alpha$ Tocopherol ( $\mu$ M)	$\gamma$ Tocopherol ( $\mu$ M)
Heterozygote TTP TRAMP+	$2.35 \pm 0.20$	$0.03 \pm 0.003$
Homozygote TTP TRAMP+	$0.16 \pm 0.015$	Below detection limit

### **Bodyweight**

The effect of the genomic constructs on bodyweight as a function of age is presented in Figure 1. As can be seen, there is virtually no effect of TTP status on bodyweight as a function of age, as the heterozygote and homozygote TTP+TRAMP mice bodyweights are indistinguishable ( $p > 0.62$ ).

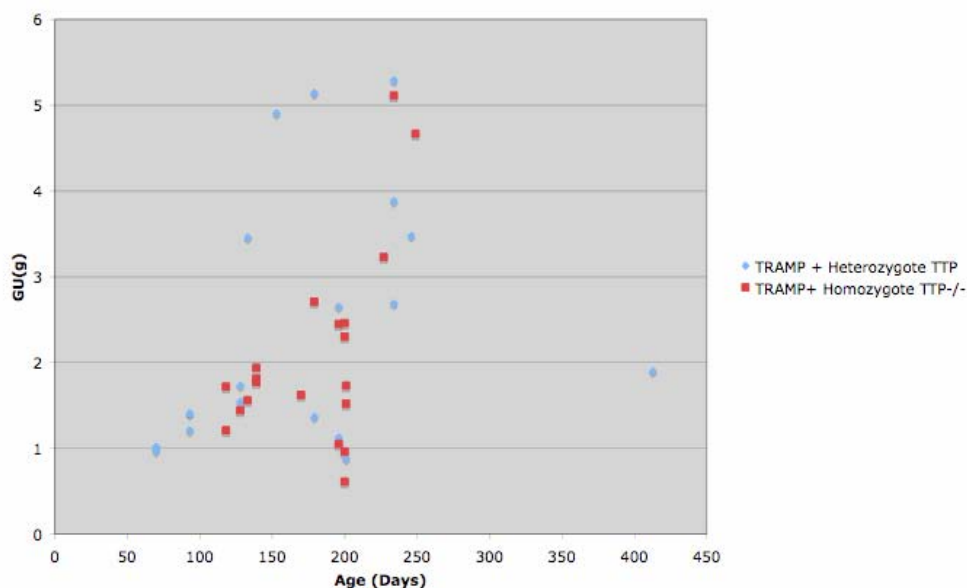
**Age versus Bodyweight by TTP status**



### **Sex pluck weight**

The effect of the genomic constructs on sex pluck weight (prostate and seminal vesicles) weight as a function of age is presented in Figure 2. As can be seen, there is virtually no effect of TTP status on tumor (sexpluck) as a function of age, as the heterozygote and homozygote TTP+TRAMP mice tumor (sexpluck) are indistinguishable.

**Age versus Sex Pluck by TTP status**



Statistical analysis of the sex pluck weight upon grouping by TTP status showed no statistically significant differences ( $p > 0.50$ ) between the TTP -/- and the TTP +/- mice. There were however statistically significant differences ( $p < 0.005$ ) between TTP -/- and

TTP+/- mice and the sex pluck weights of nonTRAMP TTP -/- mice (n = 7) (3.58 versus 3.37 versus 1.12) when analysis was restricted to sex pluck weights using a limited age range distribution. The restriction in ages compared was necessary as the nonTRAMP TTP -/- were sacrificed at a restricted age range (199±2 days old; mean ± SD) compared to the TTP/TRAMP mice.

### **Pathology and histopathology**

Very limited both in numbers and types of pathology were examined given the preliminary nature of the project and the extended period of time required to allow tumor development. Upon dissection, tumors presented as enlarged GU with a typical spherical gross appearance. There was significant hypertrophy of the seminal vesicles with multiple patches of enlarged prostate tissue evident with occasional areas of necrosis in the older mice[25]. One mouse's tumor was noted to have developed its own blood supply. Histopathology of selected mice GU also revealed the normal and anticipated range of histopathologic findings that have been documented as present in C57/bl6 x C57/bl6 TRAMP crosses[26].

### **Conclusions**

The results strongly suggest that a reduction of plasma (and presumptively tissue, though unmeasured in this study) tocopherol achieved by manipulating the levels of TTP by breeding TTP knockout animals with TRAMP mice does not accelerate the course of prostate tumor formation. The results demonstrated that heterozygote and homozygote TTP had the same sex pluck size and growth rate. These results are unexpected as it was anticipated that lowering plasma tocopherol in a stepwise fashion with heterozygote TTP knockout and then homozygote TTP knockout would accelerate tumor growth in a stepwise fashion with the intermediate tocopherol level producing a tumor production above that seen in TRAMP and TTP homozygote producing a much more drastic decline in tocopherol with resulting added increment of tumor production. The results clearly contradict this as they show no relationship between tumor developments as assessed by sex pluck weight between the different groups tested here. In addition comparison of the results documented here to those reported by Suttie and coworkers shows that the sex pluck weight increases are comparable to those obtained in their control C57/BL6 x C57/BL6 TRAMP crosses[22]. One potential ramification of these findings is the suggestion that tocopherol levels achieved on a control diet in the TRAMP model of Suttie and coworkers are already below that where tocopherol exerts an inhibitory effect on tumor formation. These results also suggests that as lowering plasma tocopherol does not accentuate tumor production, the control points for tumor production are unaffected by low tocopherol, and are already maximally turned on in the TRAMP mice. Of note concerning the model, the tocopherol model as used does not in fact represent tocopherol deficiency as would be produced by removing tocopherol from the diet but represents the decline in only the parent tocopherols tissue and plasma levels. That is it would seem likely that the metabolites of the parent compounds, would if anything increase in the face of the absence of TTP which is not the case with tocopherol deficiency produced by reduced tocopherol intake. This difference in combination with the absence of any differential effects between the TTP hetero and homozygotes suggests that the contention of Azzi and coworkers that carboxyethyl hydroxychroman metabolites are as effective as their vitamin precursors to inhibit PC-3 growth by specific down-regulation of cyclin expression [27] may not be applicable in vivo. However caution should be taken in pushing these preliminary results too far. One concern is that the numbers of animals are relatively small and the potential for missing subtle effects is elevated. Based on their experience Hurwitz and coworkers have suggested that <25 mice per treatment cohort would be required to identify a statistically significant 15 percent difference in mean tumor size within an 80% confidence interval [25]. This requirement for large cohorts in the TRAMP model is a result of the sizable stochastic differences in rates of tumor growth and differing characteristics of tumor formation that have been documented between between the C 57BL/6 T RAMP x FVB/N (TRAMPF1) mice and the pure C57BL/6 TRAMP and differences that might be expected when other mice are bred with TRAMP.

In summary, the data obtained suggest that tocopherol levels as established by TTP activity are not able to affect prostate cancer in a chow fed TRAMP mouse model. This raises questions regarding the possible effectiveness of tocopherol supplementation as treatment strategy for prostate cancer. Possible next steps might include direct supplemental feeding studies using both different tocopherols as well as these tocopherols' metabolites in a TRAMP model to assess the role of the various tocopherols themselves versus their metabolites in the development and progression of TRAMP induced prostate tumors.

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### PUBLICATIONS (from 87 total)

1. 1994 Tinker LF, Davis PA, and Schneeman BO. Prune fiber or pectin compared with cellulose lowers plasma and liver lipids in rats with diet-induced hyperlipidemia. *Journal of Nutrition* 124:31-40.
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